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Chapter 8

Depressive and anxiety disorders and short leukocyte telomere length: mediating effects of metabolic stress and lifestyle factors

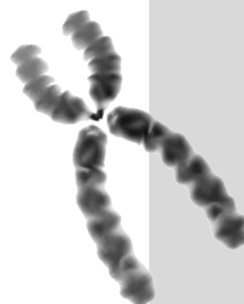
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Abstract

Background – Depressive and anxiety disorders are associated with shorter leukocyte telomere length (LTL), an indicator of cellular aging. It is, however, unknown which pathways underlie this association. This study examined the extent to which lifestyle factors and physiological changes such as inflammatory or metabolic alterations mediate the relationship.

Methods – We applied mediation analysis techniques to data from 2750 participants of the Netherlands Study of Depression and Anxiety. LTL was assessed using qPCR. Independent variables were current depressive (IDS-SR) and anxiety (BAI) symptoms and presence of a depressive or anxiety disorder diagnosis based on DSM-IV; mediator variables included physiological stress systems, metabolic syndrome components and lifestyle factors.

Results – Short LTL was associated with higher symptom severity ($B=-2.4$; $p=.002$) and current psychiatric diagnosis ($B=-63.3$; $p=.024$). C-reactive protein, interleukin-6, waist circumference, triglycerides, high-density lipoprotein cholesterol and cigarette smoking were significant mediators in the relation between psychopathology and LTL. When all significant mediators were included in one model, the effect sizes of the relationships between LTL and symptom severity and current diagnosis were reduced by 36.7% and 32.7%, respectively, and the remaining direct effects were no longer significant.

Conclusions – Pro-inflammatory cytokines, metabolic alterations and cigarette smoking are important mediators of the association between depressive and anxiety disorders and LTL. This calls for future research on intervention programs that take into account lifestyle changes in mental health care settings.

Key words – Depression; Anxiety Disorder, Telomere Length, Cellular Aging; Lifestyle; Metabolic Syndrome; Physiological Stress; Mediation

1. Introduction

Persons with a depressive or anxiety disorder have shorter telomeres, the non-coding DNA-protein complexes that cap chromosomal ends and protect DNA from damage ¹. Telomere length is considered a marker of cellular age since it shortens with every cell division and is strongly related to chronological age ² and to the incidence of various aging-related somatic diseases ^{3;4}. The association between depressive and anxiety disorders with shorter leukocyte telomere length (LTL), including a dose-response effect ⁵, has now been established by a large body of research, summarized in a recent meta-analysis including 25 studies with 21,040 participants (effect size: $r=-.12$, $p<.001$) ⁶. However, while several explanations have been proposed, the pathways and mechanisms underlying the association have not been systematically examined and therefore remain unclear.

One of the most frequently suggested pathways is that physiological changes associated with depression and anxiety lead to cellular damage, including shortened LTL. Persons with depression or anxiety disorder have higher levels of pro-inflammatory cytokines such as interleukin (IL)-6 and C-reactive protein (CRP) ⁷⁻⁹, a dysregulated hypothalamus-pituitary-adrenal (HPA)-axis ^{10;11} and impaired autonomic nervous system (ANS) functioning ^{12;13}. Further, depressive and anxiety disorders are associated with metabolic syndrome alterations such as dyslipidemia and abdominal obesity ¹⁴. These alterations all together might impact LTL and have accumulative detrimental effects ¹⁵. An additional possible explanation is that persons with depressive or anxiety disorders have unhealthier lifestyles: they are more likely to smoke, drink alcohol and are less physically active ^{16;17}, which may in turn impact LTL.

LTL correlates with several of the alterations found in persons with depression and anxiety disorder: shorter LTL was found to be associated with high levels of inflammatory markers, oxidative stress, catecholamine concentrations ^{18;19} and metabolic alterations ^{20;21}. Short LTL is also related to unhealthy lifestyle: several studies found cross-sectional associations with excessive alcohol consumption ²², smoking ²³, physical activity and increased body weight ²⁴. Physiological alterations and unhealthy lifestyle factors have thus been associated with both LTL and with depressive and anxiety disorders, making them eligible candidates to be mediating variables.

To shed more light on mechanisms underlying the association between LTL and depressive and anxiety disorders, we aimed to examine whether physiological stress system markers, metabolic alterations and lifestyle factors mediated this relationship using cross-sectional data from the large-scale observational Netherlands Study of Depression and Anxiety (NESDA). Previous analyses in this sample confirmed associations between LTL and major depressive and anxiety

disorder^{5;25}, which were strongest for subjects with the most severe and chronic symptoms, indicating a dose-response effect⁵. Other research in NESDA showed that shorter LTL was significantly associated with physiological stress including higher CRP, IL-6, heart rate (HR), cortisol awakening response¹⁵ and metabolic syndrome alterations²⁶. In NESDA we had other measures of HPA-axis, such as evening levels, diurnal pattern and dexamethasone suppression test. However, they were not associated with TL as reported before¹⁵.

In order to better understand potential mediating mechanisms that underlie increased cellular aging in persons with depression and anxiety, and to give directions to future experimental studies, the present study applied mediation analyses to test the extent to which physiological and metabolic stress markers and aspects of unhealthy lifestyle explain the association of LTL with symptom severity and depressive and anxiety disorder diagnosis.

2. Methods

2.1. Study sample

Data are from the baseline assessment of the Netherlands Study of Depression and Anxiety (NESDA), an ongoing longitudinal cohort study examining the course and consequences of depressive and anxiety disorders, as described in more detail elsewhere²⁷. The NESDA sample consists of 2981 persons between 18 and 65 years including persons with a current or remitted diagnosis of a depressive and/or anxiety disorder (74%) and healthy controls (26%). Persons were excluded when they had insufficient command of the Dutch language or a primary clinical diagnosis of other severe mental disorders, such as bipolar disorder, obsessive-compulsive disorder, post-traumatic stress disorder, severe substance use disorder or a psychotic disorder, self-reported or reported by their mental health practitioner. Participants were recruited between September 2004 and February 2007, and assessed during a 4-hour clinic visit. The study was approved by the Ethical Review Boards of participating centers, and all participants signed informed consent. For the current study, 231 subjects were excluded because of missing data on LTL, psychopathology measures or some central mediators (CRP-6, IL-6 or metabolic syndrome components), leaving 2750 subjects for the main analysis.

2.2. Measurements

2.2.1. Leukocyte telomere length

Fasting blood was drawn from participants in the morning around 8:30AM, and blood samples were stored in a -20°C freezer afterwards. As described before in more detail^{5;15}, leukocyte telomere length (LTL) was determined at the laboratory of Telomere Diagnostics, Inc. (Menlo Park, CA, USA), using quantitative polymerase chain reaction²⁸. Telomere sequence copy number in each subjects' sample (T) was compared to a

single-copy gene copy number (S), relative to a reference sample. The efficiencies of standard curve and the subject samples were similar for T and S, thereby providing a desirable precision. We converted T/S ratios to base pairs (bp) with the following formula: $bp = 3274 + 2413 * T/S$.

2.2.2. Depressive and anxiety disorder measurements

Earlier associations with LTL showed a dose-response effect indicating that persons with the most severe symptoms of depression and anxiety had the shortest LTL^{5;25}, therefore, our primary analyses focused on possible mediators in the relationship between LTL and symptom severity. Severity of depressive symptoms in the past week was assessed with the 30-item Inventory of Depressive Symptoms - Self Report (IDS-SR, range 0-84)²⁹. Anxiety severity was assessed with the 21-item Beck's Anxiety Inventory (BAI, range 0-63)³⁰. In addition, the mediating effects in the relationship between LTL and presence of current depressive or anxiety disorder were examined. Presence of a DSM-IV diagnoses of depressive (major depressive disorder, dysthymia) and/or anxiety (social phobia, generalized anxiety disorder, panic disorder, agoraphobia) disorders was ascertained using the Composite Interview Diagnostic Instrument (CIDI, version 2.1)³¹ administered by specially trained research staff. Our sample included 1569 subjects with a current (i.e. within the past 6 months) diagnosis and 608 control subjects (i.e. no lifetime history of psychiatric disorders). Previous work in the NESDA sample showed that antidepressant medication did not impact the psychopathology – LTL association, therefore medication variables were not included in the current study.

2.2.3. Physiological stress systems

As potential mediators, we selected physiological markers that within our sample have shown to be associated with LTL: c-reactive protein (CRP) and interleukin-6 (IL-6) as markers of inflammation, salivary cortisol awakening response as marker of hypothalamic-pituitary-adrenal (HPA)-axis sensitivity, and heart rate (HR) as a marker of autonomic tone dysregulation¹⁵. Circulating plasma levels of CRP and IL-6, from 8:00-9:00 AM blood draws, were assessed with high sensitivity enzyme-linked immunosorbent assays, as described before³². For the ANS measurements subjects were wearing an ambulatory electro- and impedance cardiogram system that recorded HR, an indicator of combined sympathetic and parasympathetic nervous system activity¹⁵. For 87 subjects with missing HR data at baseline, values from NESDA's 2-year follow-up assessment were used in order to maintain an optimal sample size (correlation baseline and 2-year HR: $r = .705$; $p < .001$). To examine HPA-axis hyperreactivity, subjects were instructed to collect saliva samples at home on a regular (preferably working) day at four time points: at awakening, and 30, 45 and 60 minutes later³³. We calculated the area under the curve with respect to the increase (AUCi), a measure of the dynamic of the cortisol awakening response³⁴. Of the entire cohort, a subsample of 1124 subjects had missing AUCi data due to non-compliance to

the data collection protocol, and therefore, analyses with AUCi as a mediator were done in a smaller sample (N=1812).

2.2.4. Metabolic syndrome components

Metabolic syndrome (MetS) components that were associated to LTL were considered as mediators ²⁶; of all five MetS components, only blood pressure showed no association with LTL and was not further considered. Waist circumference was measured with a tape measure at the central point between the lowest front rib and the highest front point of the pelvis, over light clothing. HDL cholesterol, triglycerides, and fasting glucose levels were determined from fasting blood samples using routine standardized laboratorial methods. According to the standards of medical care in diabetes ³⁵, antidiabetic medication aims to lower the fasting glucose level to <7.0mmol/L. Therefore, for persons using antidiabetic medication when glucose level was <7.0mmol/L, a value of 7.0mmol/L was assigned. According to the average decline in triglycerides and increases in HDL cholesterol in fibrates trials, 0.10mmol/L was subtracted from the HDL cholesterol level and 0.67mmol/L was added to the triglyceride level of persons using fibrates ³⁶.

2.2.5. Lifestyle factors

Alcohol use was expressed in number of drinks per week. Smoking was assessed by number of cigarettes per day. Physical activity was assessed using the International Physical Activity Questionnaire ³⁷, and expressed as overall energy expenditure in Metabolic Equivalent Total (MET in hours/week). Body mass index (BMI in kg/m²) was calculated as measured weight divided by height-squared.

2.3. Covariates

Sex, age and ancestry (classified as Northern-European ancestry vs. other) were assessed during the interview. The number of self-reported current somatic diseases for which participants received medical treatment (i.e. heart disease, epilepsy, diabetes, osteoarthritis, stroke, cancer, chronic lung-disease, thyroid disease, liver disease, intestinal disorders and ulcers) was counted.

2.4. Statistical analyses

Sample characteristics were described as means and standard deviations, or percentages. For non-normally distributed factors the median and interquartile range were calculated.

We first tested associations of LTL with symptom severity and depressive and anxiety disorders (together referred to as psychopathology) with regression analyses (c in **Figure 1**). Next, we applied mediation analysis to test 1) the total effect of psychopathology on possible mediators (a in **Figure 1**); 2) the effect of mediators on LTL (b in **Figure 1**); 3) the direct effect of psychopathology on LTL, corrected for a

x b (c' in **Figure 1**); 4) the indirect effect of psychopathology on LTL through the mediators ($a \times b$). All mediator variables were standardized to create comparable effect sizes. Mediation analyses were performed using the Preacher and Hayes's SPSS macro, which estimates the indirect effects of the independent variable on the dependent variable through mediator variables³⁸. This method uses bootstrap resampling procedures, in which subjects are randomly selected, with replacement, from the original sample. For each bootstrap sample the model is estimated and the parameters saved. The indirect effect is deemed significant if the 95% bootstrap percentile confidence interval did not include zero. Number of bootstraps were set at 5000. These analyses allowed us to test whether associations between psychopathology and LTL were mediated by physiological stress systems (CRP, IL-6, AUCi and HR), MetS components (waist circumference, triglycerides, HDL cholesterol, glucose) and lifestyle variables (alcohol use, smoking, BMI and physical activity), while adjusting for age, sex, ancestry and number of somatic diseases.

Mediator variables were first entered into the model separately. Subsequently, all significant mediators ($p < .05$) were entered into a multivariate model, in order to test which mediators were the main drivers in the relation between psychopathology and LTL. We calculated the change in effect (ΔB) by dividing subtracting c' from c , and dividing this residual by the c (e.g., $\Delta B = (c - c') / c$). All analyses were conducted using SPSS version 20.0 (IBM Corp., Armonk, NY, USA).

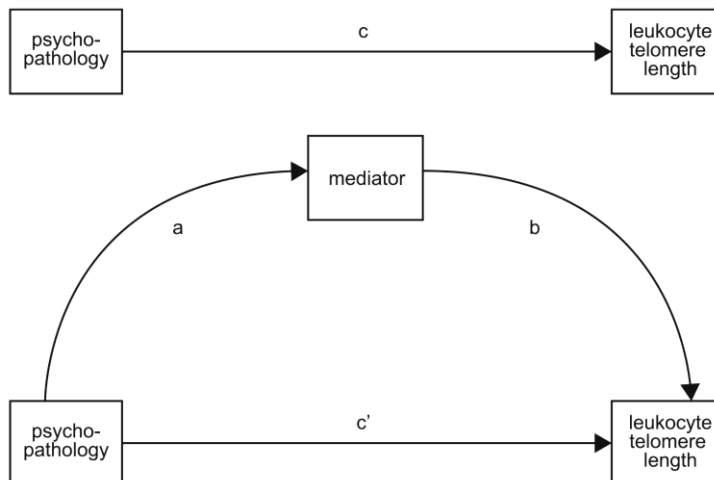


Figure 1. Illustration of the total effect of psychopathology on leukocyte telomere length (c) and a mediation design where psychopathology affects leukocyte telomere length directly (c') and indirectly ($a \times b$) through a mediator

3. Results

The mean age of the study sample (N=2750) at baseline was 42.0 years (SD=13.0, range 18-65), 67% was female and the overall majority was of North-European ancestry (**Table 1**). Average LTL was 5462bp (SD=611). The average IDS score was 8.5 (SD=7.5) for controls and 29.2 (SD=12.4) for persons with a current diagnosis. Further, controls had an average of 4.8 (SD=0.2) on the BAI and those with a current diagnosis scored 17.1 (SD=0.3). IDS and BAI scores were highly correlated ($r=0.78$; $p<.001$) and since both severity measures yielded similar results, further analyses on the BAI are presented in **Supplementary table 1**.

3.1. Associations between psychopathology and LTL (c)

Shorter LTL was associated with higher depressive ($B=-2.44$; $SE=0.81$; $p=.002$ / upper part of **Figure 2**) and anxiety ($B=-2.81$; $SE=1.07$; $p=.009$) symptom severity scores in analyses adjusted for age, sex, ancestry and chronic diseases in 2750 subjects. Further, we selected those with a current diagnosis of depressive and/or anxiety disorders (N=1569) and controls (N=608). Compared to controls, those with a current diagnosis had shorter LTL ($B=-63.30$; $SE=27.95$; $p=.024$), again adjusted for age, sex, ancestry and chronic diseases.

3.2. Effects of psychopathology on mediators (a)

Depressive symptoms were positively associated with CRP, IL-6, AUCi, waist circumference, triglycerides, cigarette smoking and BMI, negatively associated with HDL cholesterol and physical activity, and not associated with HR, glucose and alcohol use (column I, **Table 2**). Findings were similar for anxiety symptoms (**Supplementary Table 1**). Further, those with a current depressive or anxiety diagnosis showed higher CRP, AUCi, waist circumference, triglycerides, cigarettes smoking, BMI and lower HDL cholesterol and physical activity. No associations were found between current diagnosis and IL-6, HR, glucose and alcohol use (column I, **Table 3**).

3.3. Effect of mediators on LTL (b)

Shorter LTL was associated with higher CRP, IL-6, HR, waist circumference, triglycerides, glucose, and with more drinking and smoking (column II, **Table 2**). This was consistent in the analyses with controls and persons with a current diagnosis, although CRP and HR were not significantly associated with shorter LTL (column II, **Table 3**); likely due to slightly smaller sample size as directions of effects were consistent across the two tables.

3.4. Mediation of link between psychopathology and LTL (c' and a x b)

When entering separate mediators into the model with depressive symptoms and LTL, we found that higher CRP (change in direct effect: $\Delta B=-5.3\%$, column III, **Table**

2), IL-6 ($\Delta B = -4.1\%$), waist circumference ($\Delta B = -7.8\%$), triglycerides ($\Delta B = -11.9\%$) and cigarettes per day ($\Delta B = -26.6\%$), and lower levels of HDL cholesterol ($\Delta B = -3.3\%$) were significantly mediating this association (column IV, **Table 2**). When these significant mediators were combined into one multivariate model, they reduced the effect size of the association between depressive symptoms and LTL with 36.9%, and the direct effect became statistically non-significant ($B = -1.54$; $SE = 0.82$; $p = .06$; **Table 2, Figure 2**). Findings were similar for anxiety symptoms, and mediators within the multivariate model reduced the effect size of the association between anxiety symptoms and LTL with 49.1%, and rendered the direct effect of anxiety symptoms on LTL as statistically non-significant ($B = -1.43$; $SE = 1.11$; $p = .20$; **supplemental table 1**).

Table 1. Sample characteristics for the total group (N=2750), subjects with a current diagnoses (N=1569) and the healthy control group (N=608)

Demographics and somatic diseases	Total group (N=2750)	Current diagnosis (N=1569)	Healthy controls (N=608)
Age (years), mean (SD)	42.0 (13.0)	41.2 (12.3)	41.5 (14.6)
Sex (% female)	66.9	67.5	61.8
Ancestry (% North-European ancestry)	94.9	93.6	96.2
Number of somatic diseases, mean (SD)	0.6 (0.9)	0.7 (0.9)	0.5 (0.8)
Cellular aging			
Leukocyte telomere length (bp), mean (SD)	5462.1 (610.8)	5456.3 (605.7)	5514.5 (634.9)
Physiological stress systems			
C-reactive protein (mg/L), median (IQR)	1.2 (2.5)	1.3 (2.7)	1.1 (2.0)
Interleukin-6 (pg/L), median (IQR)	0.8 (0.8)	0.8 (0.8)	0.7 (0.7)
AUCi (nmol/L/hr, N=1812), mean (SD)*	2.3 (5.0)	2.4 (6.3)	1.4 (6.2)
Heart rate (beats/minute), mean (SD)	71.9 (9.6)	72.1 (9.7)	72.1 (9.0)
Metabolic syndrome components			
Waist (cm), mean (SD)	89.1 (14.0)	89.3 (14.4)	88.2 (13.5)
Triglycerides (mmol/L), median (IQR)	1.1 (0.8)	1.1 (0.8)	1.0 (0.8)
HDL Cholesterol (mmol/L), mean (SD)	1.6 (0.4)	1.6 (0.4)	1.6 (0.4)
Glucose (mmol/L), median (IQR)	5.0 (0.8)	5.0 (0.8)	5.1 (0.9)
Lifestyle factors			
Alcohol use (drinks/week), mean (SD)	6.3 (8.2)	6.1 (8.6)	6.8 (7.8)
Smoking (cigarettes/day), mean (SD)	5.0 (8.8)	6.3 (9.6)	2.7 (6.7)
Physical activity (*1000 MET-min/wk), mean (SD)	60.7 (50.8)	58.7 (51.6)	63.7 (51.3)
Body Mass Index (kg/m ²), mean (SD)	26.6 (5.0)	25.7 (5.2)	25.1 (4.6)
Psychopathology			
Depressive symptoms (IDS), mean (SD)	21.5 (14.1)	29.2 (12.4)	8.5 (7.5)
Anxiety symptoms (BAI), mean (SD)	12.1 (10.6)	17.1 (10.7)	4.0 (4.9)

Abbreviations: BAI = Beck's Anxiety Inventory; bp = base pairs; HDL = high-density lipoprotein; IDS = Inventory of Depressive Symptoms; IQR = Interquartile range; MET-min/wk = metabolic equivalent of number of calories in minutes per week; SD = standard deviation; AUCi = area under the curve with respect to the increase; * Non-imputed sample total N = 1812, N current diagnosis = 985, N controls = 427.

In models with current diagnosis, LTL and separate mediators, we found that waist circumference ($\Delta B = -4.5\%$, column III, **Table 3**), triglycerides ($\Delta B = -9.4\%$) and cigarettes per day ($\Delta B = -23.0\%$) were significant mediators (column IV, **Table 3**). When these mediators were entered into the multivariate model, only triglycerides and cigarette smoking remained significant, thereby reducing the effect size of the association between current diagnosis and LTL with 32.7%. The direct effect of

current diagnosis and LTL was no longer statistically significant ($B=-42.61$; $SE=28.30$; $p=.13$). These findings were consistent when current diagnoses of depressive or anxiety disorders were examined separately (data not shown). When the final mediation analyses were reran for men ($N=911$) and women ($N=1839$) separately, estimates of the indirect effect were in the same direction in both men and women for triglycerides (men $a \times b$: $B= -0.32$, $CI= [-0.85 - -0.02]$; women $a \times b$: $B= -0.20$, $CI= [-0.49 - -0.03]$) and for CPD (men $a \times b$: $B= -0.21$, $CI= [-0.79 - 0.31]$; women $a \times b$: $B= -0.80$, $CI= [-1.30 - -0.40]$), suggesting that sex was unlikely to moderate the effect of these mediators.

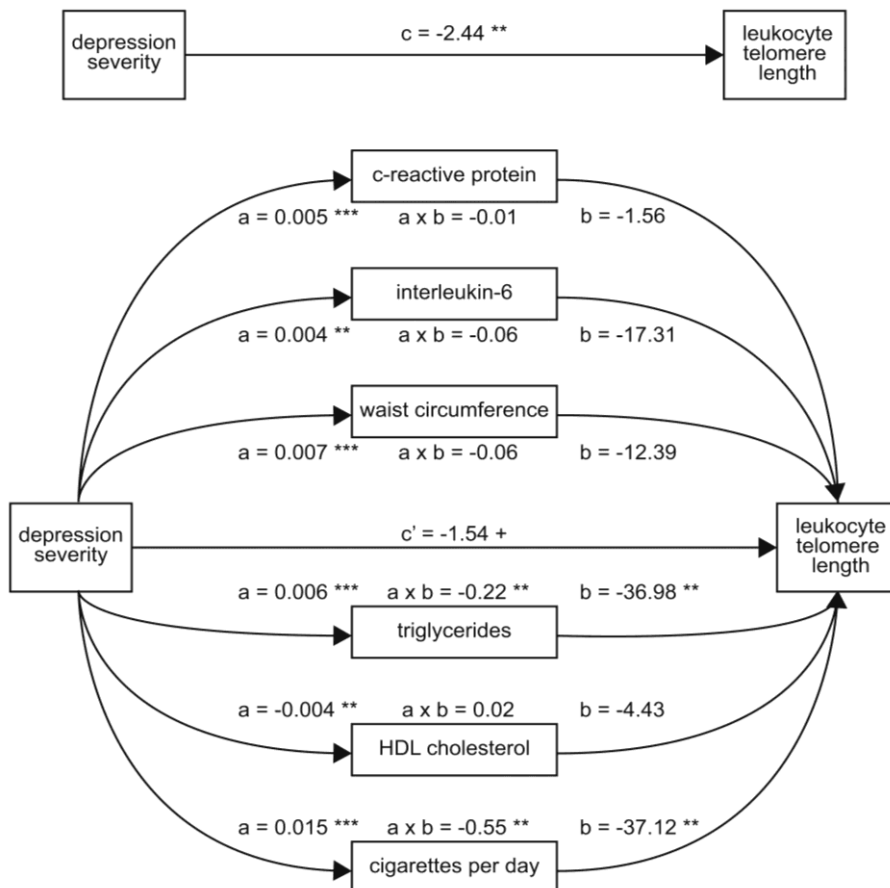


Figure 2. Direct and indirect effects of depression severity on leukocyte telomere length in a mediation design. Note. $+$ $p < .10$; $*$ $p < .05$; $**$ $p < .01$; $***$ $p < .001$; c = original effect of depression severity on LTL; a = effect of depression severity on mediator; b = effect of mediator on LTL; axb = indirect effect of depression severity on LTL; c' = direct effect of depression severity on LTL in mediation design

Table 2. Mediation analyses with separate and multiple mediators (per standard deviation increase) between depression severity (IDS) and leukocyte telomere length (LTL) (N=2750)

Total effect of IDS on LTL (c)									
B (SE) p									
2.44 (0.81) .002									
Separate mediators	I. Effect of IDS on Mediator (a)			II. Effect of Mediator on LTL (b)			III. Direct effect of IDS on LTL (c')		
	B (SE)	p	B (SE)	p	B (SE)	p	ΔB ^a	B	95% CI
Physiological stress systems									
(ln) C-reactive protein	0.005 (0.001)	<.001	-26.23 (11.21)	.02	-2.31 (0.81)	.004	-5.3%	-0.14	[-0.31 - -0.03]**
(ln) Interleukin-6	0.004 (0.001)	.008	-28.10 (11.24)	.01	-2.34 (0.81)	.004	-4.1%	-0.10	[-0.26 - -0.02]**
AUCI (N=1812)	0.003 (0.002)	<.001	-10.94 (13.37)	.41	-1.92 (1.00)	.06	-21.3%	-0.04	[-0.20 - -0.04]*
Heart rate	0.009 (0.01)	.53	-22.05 (11.25)	.05	-2.43 (0.81)	.003	-0.4%	-0.02	[-0.11 - 0.03]
Metabolic syndrome components									
Waist circumference	0.007 (0.001)	<.001	-29.31 (12.86)	.02	-2.25 (0.81)	.006	-7.8%	-0.20	[-0.41 - -0.04]**
(ln) Triglycerides	0.006 (0.001)	<.001	-48.01 (11.92)	<.001	-2.15 (0.81)	.008	-11.9%	-0.29	[-0.52 - -0.13]**
HDL cholesterol	-0.004 (0.001)	.003	22.09 (11.98)	.07	-2.36 (0.81)	.004	-3.3%	-0.08	[-0.25 - -0.001]**
(ln) Glucose	0.002 (0.001)	.87	-42.31 (12.51)	<.001	-2.44 (0.80)	.003	0%	-0.01	[-0.13 - 0.10]
Lifestyle factors									
Alcohol drinks/week	-0.001 (0.001)	.38	-26.38 (11.55)	.02	-2.48 (0.81)	.002	1.6%	0.03	[-0.03 - 0.15]
Cigarettes/day	0.01 (0.001)	<.001	-44.38 (11.30)	<.001	-1.79 (0.82)	.03	-26.6%	-0.65	[-1.01 - -0.34]**
Physical activity	-0.005 (0.001)	<.001	-9.40 (11.11)	.40	-2.49 (0.81)	.002	2.0%	0.05	[-0.05 - 0.17]
Body mass index	0.007 (0.001)	<.001	-14.83 (11.67)	.20	-2.33 (0.81)	.004	-3.7%	-0.10	[-0.28 - 0.04]
Multiple mediators									
(ln) C-reactive protein	0.005 (0.001)	<.001	-1.56 (12.96)	.90	-1.54 (0.82)	.06	-36.9%	-0.01	[-0.15 - 0.13]
(ln) Interleukin-6	0.004 (0.001)	.008	-17.31 (12.96)	.14				-0.06	[-0.21 - 0.01]
Waist circumference	0.007 (0.001)	<.001	-12.39 (15.00)	.41				-0.08	[-0.31 - 0.11]
Triglycerides	0.006 (0.001)	<.001	-36.98 (13.44)	.006				-0.22	[-0.46 - -0.07]**
HDL cholesterol	-0.004 (0.001)	.003	-4.43 (13.42)	.74				0.02	[-0.09 - 0.14]
Cigarettes/day	0.015 (0.001)	<.001	-37.12 (11.62)	.001				-0.55	[-0.91 - -0.22]**

Confidence: ΔB/C = Area under the curve for the increase HDL = High-density lipoprotein; IDS = Inventory of Depressive Symptoms; SE = standard error; ** significant based on 95% percentile interval (CI). Note: Analyses were controlled for age, sex, race and number of somatic diseases. Δ Change in Beta was not part of mediation output but calculated manually, as described in Statistical Analyses.

Footnotes: AUCI = Area under the curve with respect to the increase HDL = High-density lipoprotein; IDS = Inventory of Depressive Symptoms; ln = natural logarithm transformation; SE = standard error; ** significant based on 95% confidence interval (CI). Note: Analyses are controlled for age, sex, race and number of somatic diseases; a Change in Beta was not part of mediation output but calculated manually, as described in Statistical Analyses.

Table 3. Mediation analyses with separate and multiple mediators (per standard deviation increase) between depressive and/or anxiety disorder diagnosis and leukocyte telomere length (LTL) (N=2177)

Separate mediators	Total effect of diagnosis on LTL (c)				IV. Indirect effect of diagnosis on LTL (a x b)			
	B (SE)		p		B		95% CI	
	63.30 (27.95)		.02					
III. Direct effect of diagnosis on LTL (c')								
</								

Footnotes: AUCI = Area under the curve with respect to the increase; HDL = High-density lipoprotein; ln = natural logarithm; transformation: SE = standard error; ** significant based on 95% confidence interval (CI). Note: Analyses are controlled for age, sex, race and number of somatic diseases; a Change in Beta was not part of mediation output, but calculated manually, as described in Statistical Analyses.

Supplemental Table 1. Mediation analyses with separate and multiple mediators (per standard deviation increase) between anxiety severity (BAI) and leukocyte telomere length (LTL) (N=2750)

Total effect of BAI on LTL (c)										
					B (SE)	p				
					-2.81 (1.07)	.009				
I. Effect of BAI on Mediator (a)			II. Effect of Mediator on LTL (b)			III. Direct effect of BAI on LTL (c')			IV. Indirect effect of BAI on LTL (abd)	
Separate mediators	B (SE)	p	B (SE)	p	B (SE)	p	ΔB ^a	B	95% CI	
Physiological stress systems										
(ln) C-reactive protein	0.006 (0.002)	.001	-26.79 (11.21)	.02	-2.65 (1.07)	.01	-5.7%	-0.16	[-0.39 - -0.03]**	
(ln) Interleukin-6	0.004 (0.002)	.05	-28.69 (11.24)	.01	-2.71 (1.07)	.01	-3.6%	-0.10	[-0.30 - -0.01]**	
AUC (N=1812)	0.004 (0.003)	.09	-11.48 (13.38)	.39	-2.15 (1.41)	.13	-23.5%	-0.05	[-0.28 - -0.04]	
Heart rate	0.003 (0.002)	.18	-21.71 (11.26)	.05	-2.76 (1.07)	.01	-1.8%	-0.05	[-0.20 - -0.01]	
Metabolic syndrome components										
Waist circumference	0.008 (0.002)	<.001	-30.25 (12.84)	.02	-2.58 (1.08)	.02	-8.2%	-0.23	[-0.50 - -0.05]**	
(ln) Triglycerides	0.01 (0.002)	<.001	-48.11 (11.94)	<.001	-2.55 (1.08)	.03	-16.4%	-0.46	[-0.78 - -0.21]**	
HDL cholesterol	-0.007 (0.002)	<.001	21.83 (12.00)	.07	-2.66 (1.08)	.01	-5.3%	-0.15	[-0.39 - -0.002]	
(ln) Glucose	0.003 (0.002)	.10	-41.45 (12.53)	.001	-2.70 (1.07)	.01	-3.9%	-0.11	[-0.34 - -0.03]	
Lifestyle factors										
Alcohol drinks/week	-0.000 (0.002)	.95	-25.82 (11.55)	.03	-2.81 (1.07)	.009	0%	0.003	[-0.10 - 0.12]	
Cigarettes/day	0.02 (0.002)	<.001	-44.97 (11.41)	<.001	-1.75 (1.10)	.11	-37.7%	-1.06	[-1.65 - -0.35]**	
Physical activity	-0.004 (0.002)	.02	-8.40 (11.10)	.45	-2.85 (1.07)	.008	1.4%	0.03	[-0.05 - 0.18]	
Body mass index	0.008 (0.002)	<.001	-15.71 (11.65)	.18	-2.69 (1.08)	.01	-4.3%	-0.12	[-0.35 - -0.04]	
Multiple mediators										
(ln) C-reactive protein	0.006 (0.002)	.001	-1.52 (12.96)	.91	-1.43 (1.11)	.20	-49.1%	-0.01	[-0.18 - 0.16]	
(ln) Interleukin-6	0.004 (0.002)	.05	-17.45 (11.82)	.14				-0.06	[-0.25 - 0.01]	
Waist circumference	0.008 (0.002)	<.001	-12.13 (14.57)	.41				-0.09	[-0.34 - 0.14]	
Triglycerides	0.01 (0.002)	<.001	-35.74 (13.01)	.01				-0.34	[-0.66 - -0.10]**	
Cigarettes/day	0.02 (0.002)	<.001	-37.36 (11.66)	.001				-0.88	[-1.46 - -0.37]**	

Footnotes: AUC = Area under the curve with respect to the increase BAI = Beck's Anxiety Inventory; HDL = High-density lipoprotein; ln = natural logarithm transformation; SE = standard error; ** significant based on 95% confidence interval (CI). Note: Analyses are controlled for age, sex, race and number of somatic diseases; a Change in beta was not part of mediation output, but calculated manually, as described in Statistical Analyses.

Footnotes: AUC = Area under the curve with respect to the increase; BAI = Beck's Anxiety Inventory; HDL = High-density lipoprotein; ln = natural logarithm transformation; SE = standard error; ** significant based on 95% confidence interval (CI). Note: Analyses are controlled for age, sex, race and number of somatic diseases; a Change in Beta was not part of mediation output, but calculated manually, as described in Statistical Analyses.

4. Discussion

This large psychiatric cohort study of 2750 persons showed that the association between depressive and anxiety psychopathology and LTL was mediated by higher inflammatory markers (CRP, IL-6), less favorable metabolic profile (high waist circumference and triglycerides and low HDL cholesterol) and through increased smoking behavior. When these mediators were entered together in one model, the direct effect between symptom severity and LTL was considerably reduced.

Smoking was responsible for a substantial reduction of the direct association of LTL with psychopathology, suggesting a major role as a mediator. A relationship between smoking status and psychopathology has been well established in previous research^{39;40} and showed that persons with a lifetime depression or anxiety disorder have a twofold increased odds of being smoker^{41;42}. This might be the consequence of responses to emotional states in persons with poor distress tolerance⁴³ and high levels of neuroticism⁴⁴, which could increase vulnerability to addiction to cigarettes. Smoking is, in turn, found to be associated with alterations in hormones, such as increased levels of plasma cortisol and decreased peripheral serotonin and monoamine oxidase B activity⁴⁵, thereby possibly contributing to the persistence of psychopathology⁴⁶. Further, cigarette smoke contains reactive oxidants that induce DNA damage and activate intracellular signaling cascades leading to secretion of inflammatory markers (e.g., IL-8 and tumor necrosis factor alpha) and alterations in the adaptive T-cell immune system⁴⁷. Also, toxic components, such as nicotine, change the balance of the ANS, resulting in increased sympathetic nerve activity, including an acute increase in blood pressure and HR, but decreased heart rate variability⁴⁸. This, in combination with increased inflammation and higher leukocyte cell turnover²³, may lead to shorter telomeres. Animals models⁴⁹ and *in vitro* research⁵⁰ indeed showed that smoking was related to shorter LTL, which was further confirmed by epidemiological studies in humans^{23;51}.

Next to smoking, we found that metabolic alterations, such as abdominal obesity and dyslipidemia (high triglycerides and low HDL cholesterol), were significant mediators of the association between psychopathology and short LTL. 'Metabolic oversupply', a combination of lower energy expenditure and higher energy intake, is often seen in persons with depression and anxiety disorder^{52;53}, and is also shown to be associated with various measures of cellular aging⁵⁴⁻⁵⁶. Interestingly, waist circumference was a mediator, and BMI was not, suggesting that the role of abdominal fat specifically is more important than the role of total body fat⁵⁷. Increased adipose tissue, especially in the abdominal area, promotes inflammation, and adipocyte hypertrophy has been associated with shorter LTL²⁰. On the contrary, caloric restriction, or 'metabolic undersupply' has been shown to lead to longer telomeres and increased lifespan^{54;58}.

The association between LTL and symptom severity – but not diagnosis status – was further mediated by the inflammatory markers CRP and IL-6, although they explained a lesser part of the association than smoking and metabolic factors. Increased inflammation has often been reported in patients with depressive and anxiety disorders ⁷⁻⁹. This may be due to an unhealthy lifestyle, including physical inactivity, smoking and a bad diet, that in turn all promote inflammation, creating a deleterious vicious cycle for physical and mental health ⁵⁹. Increased adipose tissue, an active endocrine organ, might additionally release inflammatory mediators into the periphery, strengthening the notion that physiological and metabolic alterations are highly intertwined ⁵⁹. Further, inflammation is suggested to contribute to the progressive shortening of telomeres, both *in vitro* ⁶⁰, in animal models ⁶¹ and in longitudinal studies in humans ⁶²⁻⁶⁴.

In the current study, neither the HPA-axis nor ANS markers were significant mediators in the association between psychopathology and short LTL. The associations of HR and AUCi with psychopathology and LTL are possibly weaker as compared to the other mediators. Although earlier studies have shown significant associations between cortisol levels and LTL ⁶⁵⁻⁶⁹, it is hard to compare findings due to the large variety in measurement methods of cortisol (i.e. 12-hour nocturnal urine, first morning urine, or pre-post-test salivary samples). Furthermore, the associations between depression and ANS measures are conflicting in the literature. Some evidence points towards a higher sympathetic drive combined with lower vagal state among depressed persons, but the findings are inconsistent and possibly more complex due to antidepressant medication use in patients ^{12,59}.

Apart from smoking behavior, other lifestyle factors were not found to mediate the association between depressive and anxiety disorder and LTL. However, earlier studies did find that health behaviors, e.g. physical activity, dietary intake and sleep quality, influence the relationship between psychosocial stress and shorter LTL ^{70;71}. In the current study, we did not measure dietary, and whether it should be considered a mediator remains to be explored in future research. Sleep duration and insomnia were not associated with LTL in NESDA (data not shown), and were therefore not considered as potential mediators. Moreover, sleep disturbances are one of the symptoms of MDD, and it is therefore impossible to conceptually disentangle them completely from the independent variable. A possible explanation for why we did not find effects for alcohol consumption is that mainly excessive drinkers are shown to have shorter telomeres ²², while in NESDA persons with severe alcohol dependence were initially excluded at baseline. In the current study, no mediating effects were found for physical activity either, but waist circumference and dyslipidemia, plausible consequences of physical inactivity, were found to be mediators. Weight loss interventions, consisting of dietary advice and/or an increase in physical activity may be important for persons with depressive and anxiety disorders ¹⁹. Consequently, an important clinical implication that arises from this

study is the need for implementation of smoking cessation and obesity treatment in mental health care settings ^{39;72}. Although future intervention studies are needed to prove actual impact of such lifestyle interventions on cellular aging markers, preliminary evidence suggest they might be beneficial ¹⁹.

Some limitations should be taken into account when interpreting our results. As the current study has a cross-sectional design, we cannot draw conclusions regarding the direction of the effects. Future proof of concept studies may provide important evidence showing how treatment-induced change in depression impacts on LTL through the identified mediators. For instance, in randomized controlled trials treating depression with therapy of proven efficacy, it could be examined whether the improvement in depression determines a change in the mediators over repeated follow-up measures, and whether these changes parallel LTL lengthening. Some mediators might be bi-directionally associated with both psychopathology and cellular aging. Next, since we based the inclusion of mediators on earlier associations with LTL, the HPA-axis and the ANS were only represented by one measure, which may not completely capture these complex stress systems. Furthermore, telomere length has been measured only in leukocytes; however it has been shown that telomere length is strongly correlated across different tissues ⁷³. Moreover, there was no information available on dietary habits, the distribution of cell subtypes or telomerase activity. The latter would have provided valuable information regarding telomere homeostasis, as telomerase tends to lengthen the shortest telomeres more than the longer ones ⁷⁴. However, strengths of this study sample were its large size with a wide range of depressive and anxiety symptom severities and the inclusion of a variety of mediating variables.

This is the first large-scale study that tested the extent to which the association between depressive and anxiety disorders and LTL was explained by a broad range of physiological and metabolic stress markers and lifestyle factors. A substantial part of the association (33–37%) was explained by cigarette smoking, waist circumference and triglyceride levels, rendering the direct effect between psychopathology and LTL non-significant. Multifaceted interventions should target these mediators in the mental health setting, in order to break the downward cycle of psychopathology and physiological and metabolic alterations, and the eventual detrimental impact on somatic health.

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